



Association between *BRAF* mutant classification and the efficacy of pemetrexed-based chemotherapy in Chinese advanced non-small cell lung cancer patients: a multicenter retrospective study

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Background: *BRAF* mutation plays a rare but aggressive oncogenic role in non-small cell lung cancer (NSCLC) patients. The controversy of first-line chemotherapy in patients with different *BRAF* mutations exists. Here, we identified 41 stage IIIB/IV NSCLC patients with *BRAF* mutation from 3,669 NSCLC patients by next-generation sequencing (NGS) testing of ctDNA in plasma or tumor tissues.

Methods: Kaplan-Meier survival curves were used to compare the prognostic difference of progression-free survival (PFS) and overall survival (OS) in different classes of *BRAF* mutations. Multivariate Cox proportional-hazards regression was used to determine the hazard ratio (HR) of different prognostic factors in survival.

Results: A total of 40 stage IIIB/IV NSCLC patients with *BRAF* mutation were further divided into four groups according to the updated functional classification of *BRAF* mutations, 56.1% (23/41) of class 1, 12.2% (5/41) of class 2, 12.2% (5/41) of class 3 and 19.5% (8/41) of others. The median PFS of patients after first-line pemetrexed-based chemotherapy was longer than other regimens of chemotherapy (7.0 vs. 4.0 months, $P < 0.001$). The patients with class 1 *BRAF* mutation treated with pemetrexed-based first-line chemotherapy had a better OS than other regimens of chemotherapy (30 vs. 22 months, $P < 0.001$). A significant improvement of OS was observed in patients with class 1 *BRAF* mutation than other groups (25 vs. 12, 15 and 14 months, $P < 0.0001$). Multivariate analysis showed that first-line pemetrexed-based chemotherapy was associated with better PFS and OS (HR = 0.16 and 0.31, respectively; $P < 0.001$ and 0.02, respectively), as well as improved OS in patients with class 1 *BRAF* mutation than other classes (HR = 2.15, $P < 0.001$).

Conclusions: Pemetrexed-based regimen could be considered as first-line chemotherapy in advanced NSCLC patients with *BRAF* mutants when target therapy is unavailable, especially in patients harboring class 1 mutations compared with other classes.

Keywords: BRAF mutation; pemetrexed; non-small cell lung cancer (NSCLC); chemotherapy

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Introduction

Lung cancer has always been the leading cause of cancer death in all populations worldwide (1). Conventional chemotherapies used to be the cornerstone for treatment in advanced non-small cell lung cancer (NSCLC) cancer patients (2). During the past three decades, the discovery of driver genes and target therapies have significantly improved the outcome in oncogenic-addictive NSCLC patients (3,4). *BRAF* was proto-oncogene encoding a serine/threonine-protein kinase which promotes cell proliferation and survival (5). *BRAF* mutations are commonly seen in melanoma (6) and papillary thyroid carcinoma (7) and also played as one of the major oncogenic drivers occurring in 2–4% NSCLC patients (5,8). *BRAF* mutations are mainly localized in the kinase domain and about 50–80% of them are V600E mutation (9,10). However, the prognostic value of V600E mutation remains unclear (9,11) and non-V600E mutated cases showed distinctive and complicated characteristics from V600E mutant carriers (9).

Based on the increasing knowledge of the biological activation mechanism and response of inhibitors in NSCLC patients with *BRAF* mutations, a functional mutation classification system has been established recently (12,13). In detail, class 1 mutants refer to the *RAS*-independent kinase-activating V600 monomers; class 2 mutants refer to the *RAS*-independent kinase-activating dimers which are resistant to vemurafenib; class 3 refer to the *RAS*-dependent kinase-inactivating heterodimers. Previous data showed that the advanced NSCLC patients with *BRAF* mutation (18 cases with V600E and 18 cases with non-V600E) had similar OS compared with patients without *BRAF* mutation, however, V600E-mutated patients tended to have shorter PFS after platinum-based chemotherapy compared with non-V600E-mutated patients (4.1 vs. 8.9 months; $P=0.297$) (14). Increasing data support the recommendation of *BRAF* and downstream pathway targeted therapy in advanced NSCLC patients with V600E mutation (15). However, the prognosis and treatment recommendation for patients with non-*BRAF* V600 mutations remains controversial (16,17). *BRAF* inhibitor in the combination of downstream targets such as MEK inhibitor may be

better than monotherapy by reducing the drug-resistance in patients with non-*BRAF* V600 mutations (18).

Although target therapy should be the promising first-line treatment for patients with *BRAF* mutations, most of them still have to accept chemotherapy in conditions of inaccessible to target agents or progression from target therapy (19). Besides, the advance of molecular testing method could help us to explore more non-V600E mutations which would potentially contribute to the biological development of disease (20). Recently, results from one large cohort study from France showed that no clear evidence supports the influence of *BRAF* status in outcomes of patients treated with chemotherapy and patients treated by the first-line taxane had the poorest PFS (19). In this study, we aimed to explore the clinical value of the functional classification of *BRAF* mutations and optimizing the selection of chemotherapy in this cohort study. We present the following article in accordance with the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/tcr-20-480>).

Methods

Patients

We conducted a multicenter, retrospective study involving several hospitals in China between May 2014 and May 2019. The genetic alterations results obtained from 3,669 plasma or tissue samples of NSCLC patients who underwent the next-generation sequencing (NGS) detection, and IIIB-IV with *BRAF* mutations were 41 cases. Histology subtyping was determined according to the 2015 World Health Organization classification. Tumor stage was based on the 7th edition of the Lung Cancer Staging system from the American Joint Committee on Cancer. Age, smoking status, East Cooperative Oncology Group performance status, histology, disease stage, brain or bone metastasis, and molecular information were documented at first diagnosis. All clinical information, including diagnosis, treatment, and clinical outcome, was collected through the system and confirmed by local professional oncologists. Patients were followed from the date of diagnosis until the

date of death from all causes or last approachable follow-up. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST v1.1) (21). The treatment response was evaluated by CT scans at the baseline of initial therapy and every 6 weeks thereafter. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Chinese Academy of Sciences University Cancer Hospital (Zhejiang Cancer Hospital) Ethics Committee (No.: IRB-2020-188) and written informed consent was obtained from each patient to use the clinical data for research before the medical intervention started.

Molecular detection

Targeted region capture combined NGS was performed for the 41 NSCLC patients. Genomic DNA sequencing libraries were prepared using the protocols recommended by the TruSeq DNA Library Preparation Kit (Illumina, San Diego, CA, USA). For samples close to the minimum input requirement, additional pre-capture polymerase chain reaction cycles were performed to generate sufficient product for hybridization. The libraries were hybridized to custom-designed probes (Integrated DNA Technology, Coralville, IA, USA) including all exons of 170 genes and selected introns of anaplastic lymphoma kinase, RET, and ROS1 for the detection of genomic rearrangements. DNA sequencing was performed on a HiSeq3000 sequencing system (Illumina) with 2×75 bp paired-end reads. The reads were aligned to the human genome build GRCh37 using BWA (a Burrows-Wheeler aligner). Somatic single nucleotide variant and indel calls were generated using MuTect and GATK, respectively. Somatic copy number alterations were identified with CONTRA. Genomic rearrangements were identified by software developed in-house to analyze chimeric read pairs.

Statistical analyses

Kaplan-Meier curves and the two-sided log-rank test were used for univariate survival analyses. The Cox proportional hazards model was used to complete the uni- and multivariate survival analyses with the hazard ratio (HR) and corresponding 95% confidence interval. Progression-free survival (PFS) was defined as the time from the date of initial treatment to the date of systemic progression or death or censored at the date of last follow up, whichever came first to trigger the event. Significance between groups

was defined as P values <0.05. Statistical analyses were performed using the R software/environment (URL <http://www.R-project.org>).

Results

Clinicopathologic characteristics

We identified 66 NSCLC patients with *BRAF* mutation from 3,669 NSCLC patients, and 41 patients with advanced NSCLC with *BRAF* mutation were enrolled in this study (Figure 1). There were 36 males and 5 females, with a median age of 69 years (range, 34–83 years). The smoking history was divided into former or current (n=4) and never (n=37).

***BRAF* mutations**

Forty-one patients with IIIB/IV stages of NSCLC had *BRAF* mutations. The *BRAF* mutants in this study were classified into four groups according to reference (22), included class 1 [n=23 (56.1%)], class 2 [n=5 (12.2%)], class 3 [n=5 (12.2%)], and others [n=8 (19.5%)] (Table 1, Figure 2). Eight patients were stage IIIB and 33 were stage IV according to the IASLC classification of lung adenocarcinoma. The detailed characteristics are listed in Table 2. Collectively, the frequency of *BRAF* mutants included 97.6% (40/41) point mutations and 2.4% (1/41) missense mutation. The concurrent oncogenic alterations included *EGFR* (n=3), *KRAS* (n=1), *EML-ALK* (n=1) (Figure 2). The baseline clinic-genetic characteristics and chemotherapy in all patients and comparison among the four groups of patients were shown in Tables 2 and 3, respectively. No significant difference existed concerning age, gender, ECOG PS, clinical stage at diagnosis, smoking status, brain and bone metastasis, concurrent oncogenic mutations, and first- and second-lines of pemetrexed-based chemotherapy among four groups (Table 3).

First-line chemotherapy

All patients received a first-line chemotherapy regimen, including pemetrexed/platinum (n=13), pemetrexed monotherapy (n=2), paclitaxel/platinum (n=3) and gemcitabine/platinum (n=19), others (n=4). All chemotherapy regimens were calculated according to the standard dose of the NCCN guideline [pemetrexed (500 mg/m² on day 1); gemcitabine

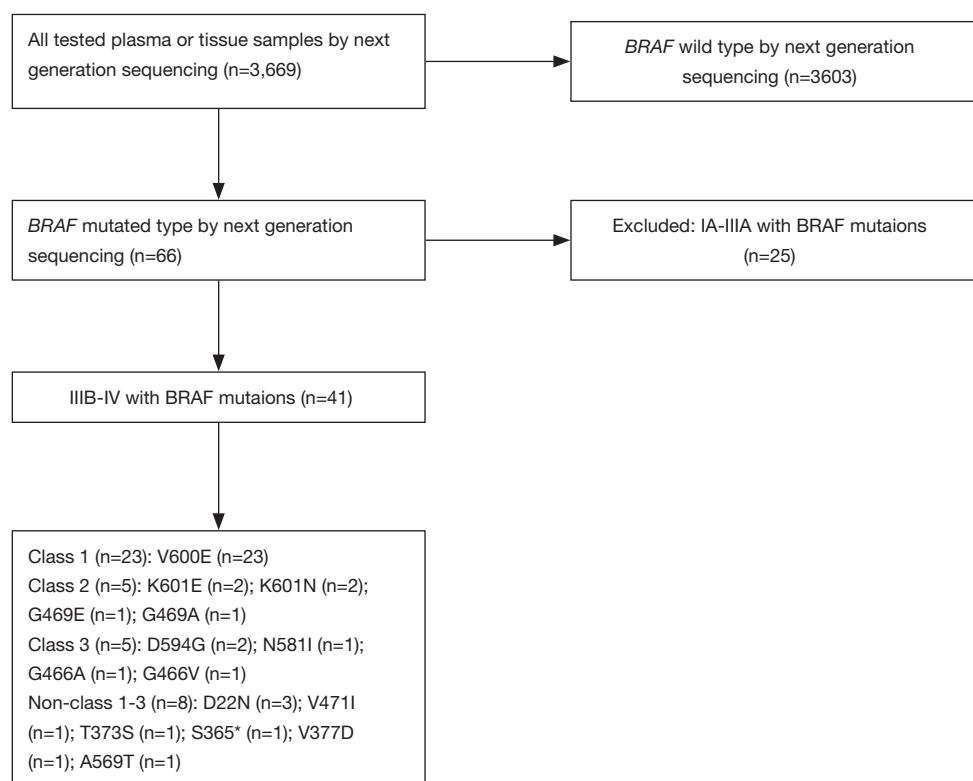


Figure 1 Flow chart of this cohort study. * represent Amino acid loss.

Table 1 *BRAF* mutations included in each functional class

Class	<i>BRAF</i> mutations
Class 1	V600E/L/D/K/M/R
Class 2	P367L/S, E451Q, G464V/E/A, G469A/V/R/S, L485W, N486_A489delinK, N486_P490del, E586K, L597Q/R/S/V, T599T/S/I/K, K601E/N/T, K601_S602delinsNT, A712T, KDD, fusions
Class 3	D287H, V459L, G466V/E/A, S467L, G469E, N581S/I/T, D594A/G/H/N, F595L, G596D/R

(1,000–1,250 mg/m² on days 1 and 8); paclitaxel (175 mg/m² on day 1); carboplatin (AUC =5 on day 1); and cisplatin (75 mg/m² on day 1)]. Of the 41 patients, 2 received chemotherapy in combination with bevacizumab and 3 received icotinib after first-line chemotherapy. The ORR and DCR of pemetrexed-based chemotherapy were 33.3% (5/15) and 53.3% (8/15), respectively. The ORR and DCR of the other chemotherapy regimens were 26.9% (7/26) and 42.3% (11/26), respectively. The median PFS (mPFS) for the 15 patients who received pemetrexed-based chemotherapy was 7.0 months, while the mPFS of the 26 patients who received other chemotherapy regimens was 4.0 months [P<0.001, HR =0.31 (95% CI, 0.15–0.61);

Table 4, Figure 3A]. Of 23 patients with class 1 *BRAF* mutations, the pemetrexed-based chemotherapy-treated patients (n=10) have better mOS than non-pemetrexed-based chemotherapy-treated patients (n=13) was significantly different [30.0 vs. 22.0 months, P<0.001, HR =0.29 (95% CI, 0.12–0.73); Figure 3B]. Overall survival of patients with class 1 *BRAF* mutation treated with pemetrexed-based or other chemotherapy regimens as first-line chemotherapy (30 vs. 22 months, P=0.0002) (Figure 3C).

Overall survival (OS)

The median OS (mOS) of the 41 patients IIIB/IV NSCLC

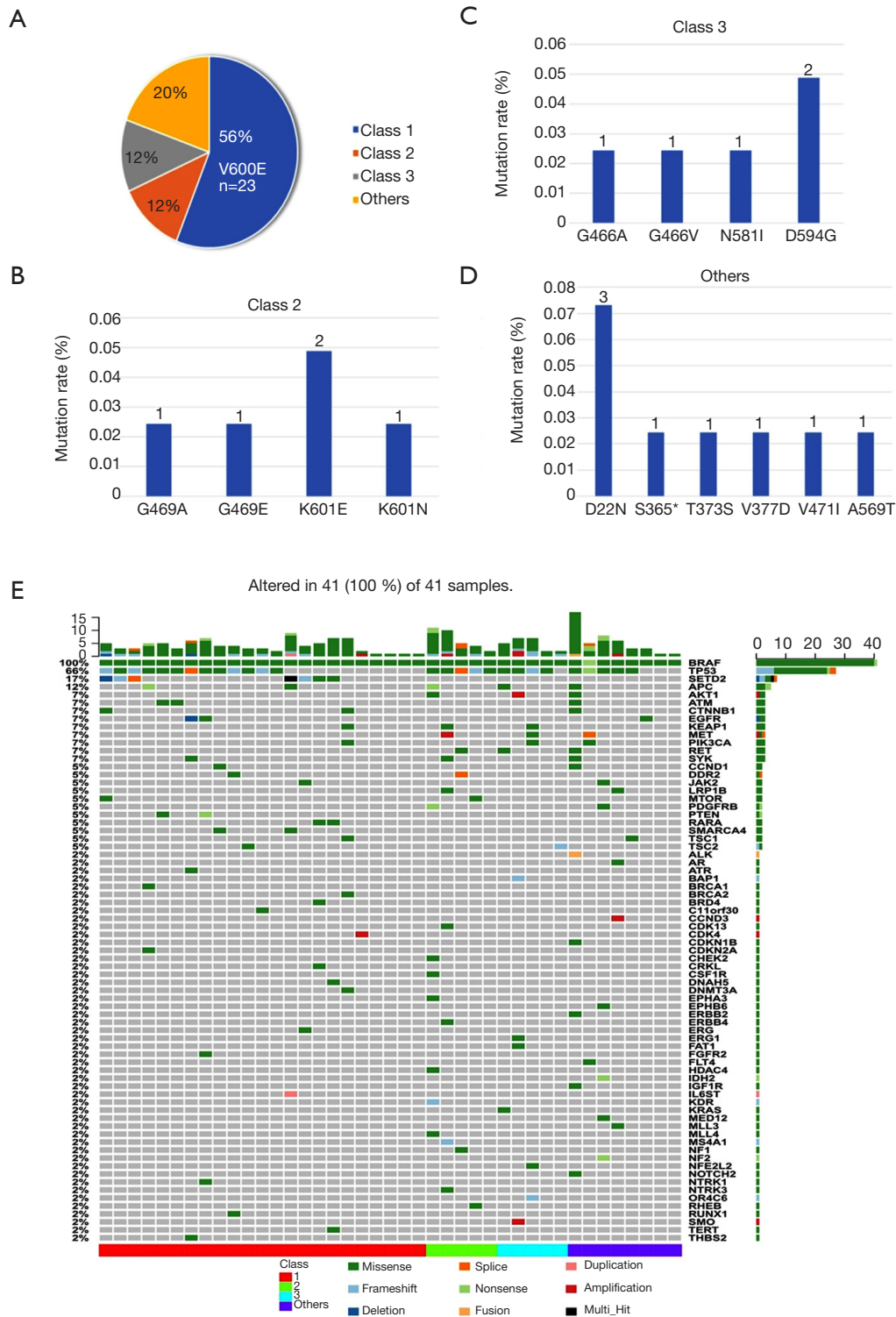


Figure 2 Distribution of V-RAF murine sarcoma viral oncogene homolog B (*BRAF*) mutants in 41 advanced non-small cell lung Cancer (NSCLC) patients according to different classes by functional classification [(A) mutation proportion of each subtype, (B) number of Class 2, (C) number of Class 3, (D) number of others]. Heatmap showed the next-generation sequencing results for mutation spectrum of *BRAF* mutants in all patients (E). * represent Amino acid loss.

Table 2 Clinical characteristics of the study population (n=41)

Characteristic	Number (%)
Gender	
Male	36 (87.8)
Female	5 (12.2)
Age (years)	
Mean	66.1
≤60	7 (17.1)
>60	34 (82.9)
Smoking status	
No	37 (90.2)
Yes	4 (9.8)
Histology	
Adenocarcinoma	29 (70.7)
Non-adenocarcinoma	12 (29.3)
ECOG PS	
0–1	33 (80.5)
≥2	8 (19.5)
Clinical stage	
IIIB	8 (19.5)
IV	33 (80.5)
Brain metastases before treatment	
No	38 (92.7)
Yes	3 (7.3)
Bone metastases before treatment	
No	34 (82.9)
Yes	7 (17.1)
BRAF-mutant group	
Class 1	23 (56.1)
Class 2	5 (12.2)
Class 3	5 (12.2)
Others	8 (19.5)
Concurrent oncogenic mutations	
No	36 (87.8)
Yes	5 (12.2)
First-line chemotherapy	
Pemetrexed-based	15 (36.6)
Non-pemetrexed	26 (63.4)
Second-line chemotherapy	
Pemetrexed-based	3 (7.3)
Non-pemetrexed based	38 (92.7)

patients was 22.0 months (*Figure 4A*). A significant improvement of OS existed in patients with *BRAF* mutation of class 1 compared with class 2, class 3 and others (n=23.0, 5.0, 5.0, 8.0; 25.0 vs. 12.0, 15.0 and 14.0 months, $P<0.0001$; *Figure 4B*). Multivariate analysis revealed that the pemetrexed-based chemotherapy and class 1 *BRAF* mutated were independent prognostic factors for OS in all patients after adjusting for known characteristic factors ($P<0.001$; *Table 4*).

Discussion

We have carried out the study to explore the efficacy of pemetrexed-based chemotherapy in advanced NSCLC patients with the different functional classification of *BRAF* mutations. Patients who treated by first-line pemetrexed-based chemotherapy had better PFS and OS than other regimens. Furthermore, patients with class 1 *BRAF* mutants had a better OS than other groups after first-line pemetrexed-based chemotherapy. As far as we know, this is the first study reported the positive association between the efficacy of pemetrexed-based chemotherapy and the functional classification of *BRAF* mutation in advanced NSCLC patients.

BRAF mutations in NSCLC have been well-known as the unique driver-gene alterations than common mutations (23). Dagogo-Jack *et al.* (24) reported a significantly longer OS for *BRAF*-mutant NSCLC patients with class 1 than other classes after first-line chemotherapy (1 vs. 2, $P<0.001$; 1 vs. 2, $P=0.023$). However, Lin *et al.* (22) found comparable OS outcomes among all classes of *BRAF* mutation by 28.6, 13.9 and 20.2 months for classes 1, 2 and 3, respectively ($P=0.585$). The controversy of those results may intratumor heterogeneity of *BRAF* mutations and response to different regimens of chemotherapy. Nevertheless, according to the available evidence, patients with class 2 and 3 appeared to have a worse outcome than patients with class 1.

Before the *BRAF* mutation story initiated from melanoma to the unique subset in NSCLC (25), conventional platinum-based chemotherapy is the cornerstone treatment in advanced NSCLC patients with *BRAF* mutations (26). Platinum-based chemotherapies were not effective in metastatic NSCLC patients with *BRAF* V600E (14). Therefore, searching for better chemotherapeutic agents become very important when target therapy is inaccessible. Pemetrexed is a multitargeted antifolate and it could be used in combination with platinum

Table 3 Comparison of clinical characteristics of BRAF-mutant groups (n=41)

Characteristic	Class 1	Class 2	Class 3	Others	P
Gender					0.58
Male	21 (91.3%)	0 (0.0%)	1 (20.0%)	2 (25.0%)	
Female	2 (8.7%)	5 (100.0%)	4 (80.0%)	6 (75%)	
Age (years)					0.54
Mean	65.1	70.8	67.4	65.3	
≤60	5 (21.7%)	0 (0.0%)	0 (0.0%)	2 (25.0%)	
>60	18 (78.3%)	5 (100.0%)	5 (100.0%)	6 (75.0%)	
Smoking status					0.79
No	20 (87.0%)	5 (100.0%)	5 (100.0%)	7 (87.5%)	
Yes	3 (13.0%)	0 (0.0%)	0 (0.0%)	1 (12.5%)	
Histology					0.11
Adenocarcinoma	16 (69.6%)	2 (40.0%)	3 (60.0%)	8 (100.0%)	
Non-adenocarcinoma	7 (30.4%)	3 (60.0%)	2 (40.0%)	0 (0.0%)	
ECOG PS					0.87
0–1	18 (78.3%)	4 (80.0%)	5 (100.0%)	6 (75.0%)	
≥2	5 (21.7%)	1 (20.0%)	0 (0.0%)	2 (25.0%)	
Clinical stage					0.21
IIIB	19 (82.6%)	2 (40.0%)	2 (40.0%)	0 (0.0%)	
IV	4 (17.4%)	3 (60.0%)	3 (60.0%)	8 (100.0%)	
Brain metastases before treatment					0.39
No	20 (87.0%)	5 (100.0%)	5 (100.0%)	8 (100.0%)	
Yes	3 (13.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Bone metastases before treatment					0.53
No	18 (78.3%)	5 (100.0%)	5 (100.0%)	6 (75.0%)	
Yes	5 (21.7%)	0 (0.0%)	0 (0.0%)	2 (25.0%)	
Co-occurring oncogenic alterations					0.61
No	21 (91.3%)	5 (100%)	4 (80.0%)	6 (75.0%)	
Yes	2 (8.7%)	0 (0.0%)	1 (20.0%)	2 (25.0%)	
First-line chemotherapy					0.26
Pemetrexed-based	10 (43.5%)	1 (20.0%)	0 (0.0%)	4 (50.0%)	
Non-pemetrexed	13 (56.5%)	4 (80.0%)	5 (100.0%)	4 (50.0%)	
Second-line chemotherapy					0.86
Pemetrexed-based	2 (8.7%)	0 (0.0%)	0 (0.0%)	1 (12.5%)	
Non-pemetrexed based	21 (91.3%)	5 (100.0%)	5 (100.0%)	7 (87.5%)	

Table 4 Results of multivariate Cox regression model in *BRAF* mutation patients

Covariant	Coefficient	Standard error	P value	HR	95% CI	
					Lower	Upper
Age (≤ 60 vs. >60)	0.99	0.57	0.99	1.01	3.27×10^{-1}	3.00
Gender (female vs. male)	0.31	0.88	0.18	0.31	5.46×10^{-2}	1.71
Smoking status (no vs. yes)	0.62	0.62	0.45	1.6	1.87×10^{-1}	2.09
Histology (adenocarcinoma vs. non-adenocarcinoma)	0.01	0.51	0.99	0.99	3.73×10^{-1}	2.73
Clinical stage (IIIB vs. IV)	0.73	0.51	0.54	1.37	2.67×10^{-1}	2.00
First-line chemotherapy (pemetrexed-based vs. non-pemetrexed)	49.22	1.39	0.005	0.02	3.23	750.62
Pemetrexed-based chemotherapy (no vs. yes)	0.00	1.54	3.93×10^{-5}	0.00	8.84×10^{-5}	0.04
<i>BRAF</i> mutant group (Class 1 vs. Class 2 vs. Class 3 vs. non-class 1–3)	1.17	0.22	9.72×10^{-8}	3.21	2.09	4.92
Co-occurring oncogenic alterations (no vs. yes)	1.88	0.86	0.46	0.53	9.87×10^{-2}	2.87

HR, hazard ratio; CI, confidence interval.

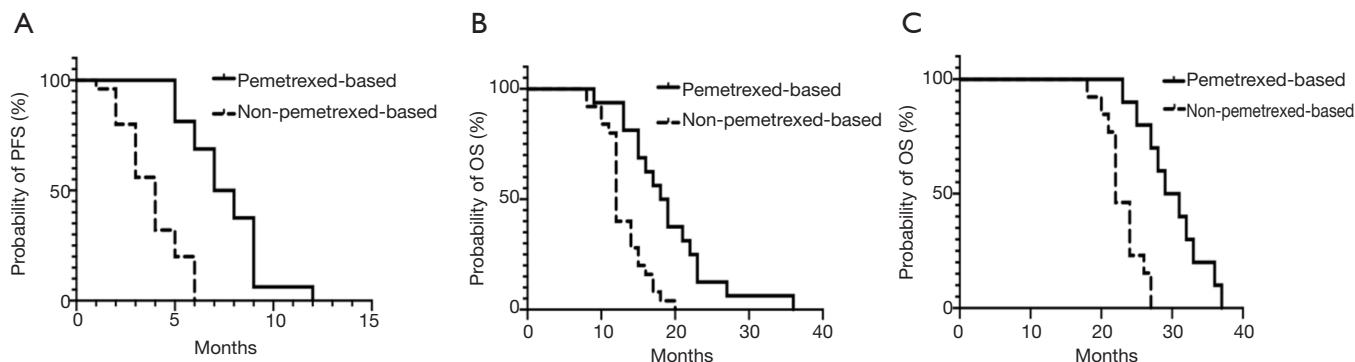


Figure 3 Survival analysis. (A) Progression-free survival of all patients with *BRAF* mutation treated with first-line pemetrexed-based or other chemotherapy (7.0 vs. 4.0 months, $P < 0.001$); (B) overall survival of patients with class 1 *BRAF* mutation treated with pemetrexed-based or other chemotherapy regimens as first-line chemotherapy (30.0 vs. 22.0 months, $P < 0.001$); (C) overall survival of patients with class 1 *BRAF* mutation treated with pemetrexed-based or other chemotherapy regimens as first-line chemotherapy (30 vs. 22 months, $P = 0.0002$).

in first-line or monotherapy for subsequent lines and as well as maintenance therapy (27). Over eight years survival benefit from pemetrexed treatment in an advanced NSCLC case with *BRAF* mutation has been reported in Japan recently (28). The higher frequency of adenocarcinoma (69.6%) in patients with class 1 *BRAF* mutation could also

indicate the potential better response to pemetrexed based chemotherapy (29). Liang and colleagues have launched interesting research on long-term pemetrexed response in NSCLC with driver oncogenes (30). Although *BRAF* mutation has not included in their research, the mechanism of significantly better survival from long-term pemetrexed

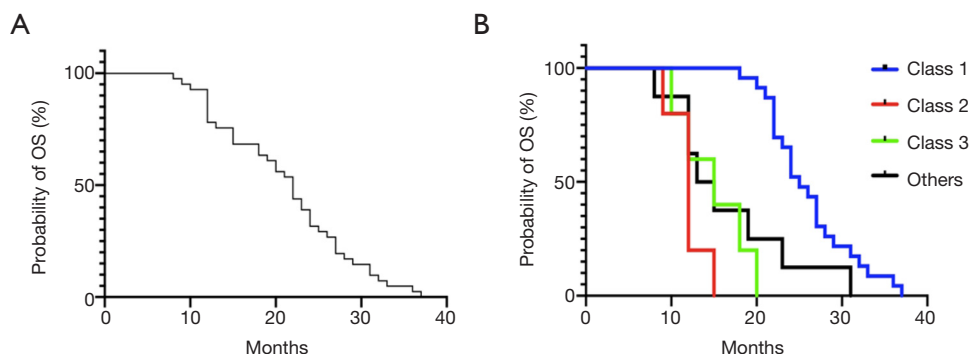


Figure 4 Survival analysis. (A) The median overall survival (OS) of the 41 patients was 22.0 months; (B) a significant improvement of OS was seen in lung cancer patients with class 1 BRAF mutation than patients with other classifications (25.0 vs. 12.0, 15.0 and 14.0 months, $P < 0.0001$).

response in oncogenic-addiction patients should be explored.

We have also explored the concurrent driver genetic alterations in this study. The advanced NSCLC patients with *BRAF* mutation have seldomly co-occurrence with other driver genes (5). Under screening by the known driver-gene panel in NSCLC, only 5 patients have been detected and three of them harboring *EGFR* mutation have received icotinib. The other two patients with *KRAS* A146T and *EML4-ALK* respectively did not receive any target therapies. Of note, two patients with class 1 *BRAF* mutation co-occurring with *EGFR* mutation but not *KRAS* consistent with mutual exclusivity existed between class 1 *BRAF* mutation and *KRAS* mutation in a previous study (22).

There are some limitations to our research. Firstly, this is a retrospective study and the enrollment of patients could be biased. However, it is not easy for us to collect patients with a rare mutation from a large cohort by NGS testing. Secondly, although the standard recommendation for patients with *BRAF* V600E mutation should be the combination of BRAF and MEK inhibitors (31), none of the patients have ever been treated by targeted therapy due to numeric reasons. We believe that the value of our research is to pursue the most available and effective chemotherapy for those patients who could not afford those expensive targeted drugs outside of clinical trials.

Conclusions

Pemetrexed-based chemotherapy treatments were more effective than other regimens in advanced NSCLC patients with *BRAF* mutations. The best clinical benefit of first-line

pemetrexed-based chemotherapy was observed in patients with class 1 *BRAF* mutants among all patients. In that case, we assumed that pemetrexed-based chemotherapy could be an alternative treatment of choice in NSCLC patients with *BRAF* mutation when the targeted therapy is unavailable. Besides, more research and workforce are warranted in this area.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Chinese Academy of Sciences University Cancer Hospital (Zhejiang Cancer Hospital) Ethics Committee (No.: IRB-2020-188) and written informed consent was taken from all the patients.

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